JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1996, p. 834–841 Vol. 34, No. 4
0095-1137/96/$04.00+0

Diagnostic Performance of Amplified *Mycobacterium tuberculosis* Direct Test with Cerebrospinal Fluid, Other Nonrespiratory, and Respiratory Specimens

GABY E. PFYFFER,[1]* PASCALE KISSLING,[1] ESTHER M. I. JAHN,[1] HANS-MARTIN WELSCHER,[1] MAX SALFINGER,[2] AND RAINER WEBER[3]

Swiss National Center for Mycobacteria, Department of Medical Microbiology, University of Zurich, CH-8028 Zurich,[1] and Division of Infectious Diseases, Department of Medicine, University Hospital, CH-8091 Zurich,[3] Switzerland, and Wadsworth Center for Laboratories and Research, New York State Department of Public Health, Albany, New York 12201-0509[2]

Received 17 November 1995/Returned for modification 20 December 1995/Accepted 23 January 1996

The Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test (MTD) was adapted to be used for cerebrospinal fluid (CSF) and a large variety of other nonrespiratory specimens. Standardized with artificially spiked dilution series of CSF, the modified MTD procedure consists of (i) increasing the amount of sample 10-fold, (ii) pretreating the specimen with a detergent, and (iii) increasing the amplification time from 2 to 3 h. Performance of MTD in a clinical mycobacteriology laboratory was tested over an extended period of time, involving a total of 322 nonrespiratory as well as 1,117 respiratory specimens from 998 patients. Results from MTD were compared with those from microscopy, culture, analysis of tuberculostearic acid by gas-liquid chromatography-mass spectrometry (CSF only), and the final clinical diagnosis. When MTD results were compared with resolved data, the sensitivity, specificity, and positive and negative predictive values for MTD were 93.1, 97.7, 90.0, and 98.5%, respectively, for nonrespiratory specimens and 86.6, 96.4, 76.8, and 98.1%, respectively, for respiratory specimens. Our data demonstrate that (i) MTD is a robust, highly sensitive and specific technique for the rapid detection of *M. tuberculosis* complex in all types of clinical specimens, (ii) there was no statistically significant difference ($P > 0.005$) in sensitivity and specificity for nonrespiratory compared with respiratory specimens, and (iii) repeating all MTDs which yield a result between 30,000 and 200,000 relative light units would help prevent a large number of false positives and, thus, enhance test specificity.

Diagnostic techniques based on amplification have the potential to increase the sensitivity for detecting mycobacteria as well as to dramatically reduce the time usually necessary to detect and identify these organisms in clinical specimens (23). Often quite demanding as far as technical equipment and operational skills are concerned, many of the protocols for detecting *Mycobacterium tuberculosis* do not, however, fit easily into a clinical laboratory's work flow (21). Since the numerous studies with clinical specimens differ, in addition, in the amplification technique, including lysing methods, target nucleic acids, primers, and the procedures used to detect amplified products, the reported sensitivities and specificities are hard to compare. To overcome these difficulties, two important techniques have recently been introduced in a kit-based format, PCR (3) and transcription-mediated amplification (8). In a large multicenter study comprising more than 5,800 respiratory specimens, an overall sensitivity of 83% and a specificity of 99% for the PCR-based technology AMPLICOR MTB (Roche Diagnostic Systems, Somerville, N.J.) were reported (25). Similarly, for more than 5,000 respiratory specimens transcription-mediated amplification (Amplified *M. tuberculosis* Direct Test [MTD]; Gen-Probe, San Diego, Calif.) yielded overall sensitivities between 82 and 97% and specificities between 97 and 100% (1, 8, 16, 19).

In contrast, there is considerably less experience with direct detection of *M. tuberculosis* in nonrespiratory specimens. Paradoxically, it is precisely the extrapulmonary situation of tuberculosis (TB), e.g., tuberculous pleuritis or lymph node TB, for which a rapid and accurate laboratory diagnosis is of prime importance, since the traditional techniques of detecting acid-fast bacilli (AFB) have their well-known limitations. Such limitations are mainly due to the small amount of bacteria normally present in these types of specimens. Clarridge et al. reported on the amplification of a 317-bp segment within the insertion element IS*6110* to detect *M. tuberculosis* in a variety of nonrespiratory specimens (2). Amplification of a 162-bp region of the genes coding for the mycobacterial antigen 85 complex (5) and nested PCR amplifying a gene sequence that encodes the 38-kDa protein of *M. tuberculosis* (17) have also been applied. Recently, some information on the performance of MTD with a limited number of approximately 150 nonrespiratory specimens became available (4, 26).

Among the extrapulmonary TB manifestations, tuberculous meningitis undoubtedly presents one of the most difficult diagnostic problems. Microscopy yields positive smears in 3 to 37% of first samples only, and culture on solid media—apart from being very slow—is similarly insensitive, since growth is observed only in 37 to 52% of cases (9). The introduction of gas-liquid chromatography-mass spectrometry (GLC-MS) into the clinical laboratory represented a breakthrough in the direct analysis of cerebrospinal fluid (CSF), inasmuch as the presence of tuberculostearic acid (*R*-10-methyl octadecanoic acid) (TBSA), a structural component of mycobacteria as well as of certain aerobic gram-positive rods (14), may indicate tuberculous meningitis (6). However, the sensitivity of this technique

* Corresponding author. Mailing address: Swiss National Center for Mycobacteria, Department of Medical Microbiology, University of Zurich, Gloriastrasse 30, 8028 Zurich, Switzerland. Phone: 41 1 257 27 86. Fax: 41 1 261 87 90.

compared with that of radiometric culture (BACTEC) remains unknown.

The purpose of the present study was fourfold. (i) In light of an urgent need for a more sensitive and rapid diagnosis in clinical situations in which only a few AFB are present, we aimed at establishing MTD for application to CSF. (ii) We also intended to adapt MTD to the large spectrum of other nonrespiratory specimens which are usually seen in a clinical mycobacteriology laboratory. (iii) We aimed at assessing the reliability of the cutoff value for both nonrespiratory and respiratory specimens. (iv) Finally, we wanted to monitor the performance of MTD in the daily routine of a clinical mycobacteriology laboratory over an extended period of time.

MATERIALS AND METHODS

Clinical specimens. Clinical specimens (n = 1,439) were obtained from patients admitted to the University Hospital of Zurich, Switzerland, and other hospitals in the metropolitan area as well as from patients consulting private physicians. Nonrespiratory specimens (n = 322) were collected for 1 year (August 1994 to July 1995); respiratory specimens (n = 1,117) were collected for 2 years (August 1993 to July 1995). All patients (n = 998) were suspected to have TB. Only upon explicit request of the physician were clinical specimens analyzed by MTD. Criteria for analysis by MTD were (i) high suspicion of TB on account of clinical and radiological findings, (ii) immunosuppression (due to human immunodeficiency virus [HIV] infection, immunosuppressive therapy, transplant, etc.), and (iii) high TB prevalence in a particular population (prisoner, drug abuser, homeless, etc.) associated with the patient. In parallel, all specimens underwent conventional TB diagnostic procedures (i.e., microscopy, culture, and identification). The bulk of specimens originated from the respiratory tract (538 sputum samples, 316 bronchial and 7 tracheal aspirates, 256 bronchoalveolar lavage [BAL] specimens) while approximately one-fourth of the specimens (22.4%) were of nonrespiratory origin (Table 1). Upon receipt, all specimens were kept at 4°C prior to processing (two workups per day).

Pretreatment of clinical specimens for culture and microscopy. Sputa and bronchial and tracheal aspirates as well as BAL specimens (<10 ml) in 50-ml plastic centrifuge tubes were adjusted to 10 ml with sterile distilled water and decontaminated with 3.16% sodium dodecyl (lauryl) sulfate–1% NaOH (SDS-NaOH) (19). BAL specimens (>10 ml), urine (50 ml), and neutralized gastric fluid (filled up to 50 ml with sterile 0.067 M phosphate buffer [pH 6.8]) were centrifuged at 3,300 × g for 15 min. The supernatant fluid was discarded, and the sediment was resuspended in 10 ml of H_2O and treated with SDS-NaOH as described above.

Lymph nodes and tissue specimens were homogenized in a Ten Broeck mortar. One hundred microliters of these homogenates as well as 100 μl of other, normally sterile nonrespiratory specimens (CSF, biopsy samples, pleural aspirates, etc.) was applied to Chocolate II agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and incubated 48 h at 36 ± 1°C. Decontamination was only performed when growth of bacteria or fungi was observed on this medium.

Pretreatment of clinical specimens for amplification. (i) Pretreatment of CSF. We first used CSF which had been artificially spiked with cells of *M. tuberculosis*. For this purpose, CSF originating from patients with suspected diseases other than TB was obtained from the Department of Clinical Chemistry, University of Zurich. CSF was homogeneously spiked with cells of *M. tuberculosis* ATCC 25618 which were removed with a loop from a Löwenstein-Jensen slant (produced in our department) and were suspended in 5 ml of phosphate buffer. The suspension was thoroughly homogenized with glass beads (Vortex) and adjusted by dilution with phosphate buffer (0.067 M, pH 6.8) to an A_{420} of 0.15, which corresponds to approximately 10^8 mycobacteria per ml (22). This was equivalent to ca. 2×10^6 CFU/ml. Tenfold dilutions (to approximately 10^1 cells per ml of CSF) were made in 5-ml sterile glass test tubes. CSF was subjected to different pretreatment protocols prior to amplification. These included (i) no pretreatment at all, (ii) centrifugation (15,000 × g, 15 min) and washing of the pellet in phosphate buffer (0.067 M; pH 6.8), (iii) centrifugation and addition of 10 μl of Tween 80 to the washed pellet, (iv) addition of 0.5 ml of 12.5% trichloroacetic acid (4°C, 30 min), and (v) treatment with SDS-NaOH. For the latter procedure, aliquots of 500 μl of CSF were mixed with 500 μl of 3.16% SDS–1.0% NaOH (19). After vortexing, the specimens were vigorously shaken for 30 min and then left in the rack for another 10 min. Five hundred microliters of 1.43% H_3PO_4 (containing 0.006% bromcresol purple as a pH indicator) were added to neutralize the specimen. After a centrifugation step (15,000 × g, 15 min) the micropellet was thoroughly washed with 500 μl of H_2O and centrifuged again (15 min). The sediment was finally resuspended in 50 μl of 0.067 M phosphate buffer (pH 6.8), mixed well, and subjected to MTD.

(ii) Pretreatment of all other types of nonrespiratory specimens. All other types of nonrespiratory specimens (except urine and gastric fluid) were also pretreated with SDS-NaOH (see above).

TABLE 1. Summary of clinical specimens analyzed by MTD and culture[a]

Origin and type of specimen	n	No. of specimens positive by:		
		MTD and culture	MTD only	Culture only
Urine	53	8	3	0
Gastric fluid	17	1	0	0
Cerebrospinal fluid	54	5	3	0
Lymph node	46	12	7	0
Other body fluids or aspirates				
Pleural	65	2	3	2
Pericardial	14	0	1	0
Vertebral	6	0	0	0
Ascites	12	0	0	0
Intervertebral disc	2	0	0	0
Douglas	1	0	0	0
Synovial fluid	2	0	0	0
Biopsy specimens				
Lung	8	0	0	0
Liver	2	0	0	0
Spleen	1	0	0	0
Skin	2	0	0	0
Pleural	4	2	0	0
Transbronchial	1	0	0	0
Mucosal	3	0	0	0
Vertebral	3	1	1	0
Disc	1	0	0	0
Ear	3	0	0	0
Pus	17	8	1	2
Pannus	2	0	2	0
Graft	3	0	0	0
Sputum	538	44	22	8
Tracheal aspirate	7	0	1	0
Bronchial aspirate	316	37	8	5
BAL	256	20	19	5

[a] Specimens from sputum, tracheal and bronchial aspirates, and BAL are classified as respiratory; all other specimens in the table are nonrespiratory. Totals for n and numbers of specimens positive by both MTD and culture, by MTD only, and by culture only for nonrespiratory specimens were 322, 39, 21, and 4, respectively. Corresponding totals for respiratory specimens were 1,117, 101, 50, and 18, respectively.

(iii) Pretreatment of respiratory, urine, and gastric-fluid specimens. Respiratory, urine, and gastric-fluid specimens were pretreated with SDS-NaOH according to the protocol described previously (19).

Culture. Clinical specimens were inoculated onto a Löwenstein-Jensen slant, onto a Middlebrook 7H10/selective 7H11 agar biplate (Becton Dickinson Microbiology Systems), and into BACTEC 12B medium (19). Solid media were read weekly, and BACTEC cultures were read twice weekly for the first 2 weeks and once weekly thereafter.

Microscopy. Microscopy was done for all clinical specimens except for most gastric fluids. Urine samples were screened microscopically only when they were submitted by a nephrologist. Smears were stained with auramine-rhodamine fluorochrome. Positive slides were confirmed by Ziehl-Neelsen staining (10).

Identification of mycobacteria. Routine biochemical methods (10, 18) and Accuprobe culture confirmation kits (Gen-Probe) were employed for the identification of isolates. Cellular fatty-acid patterns were analyzed by using GLC in conjunction with the Microbial Identification System (Microbial ID Inc., Newark, Del.) (24).

MTD. MTD was performed twice weekly with 50 μl of pretreated specimen (SDS-NaOH) according to the instructions of the manufacturer. Hybridization protection assay-positive and -negative controls were included in each series at the beginning of each run. In addition, all MTD series included a control consisting of *M. tuberculosis* ATCC 25618 as well as MTD amplification-positive and -negative controls, which were measured after the hybridization protection assay controls and at the end of each run. The cutoff value was set by the manufacturer: samples with values of ≥30,000 relative light units (RLU) were considered positive, and samples with values of <30,000 RLU were considered negative.

Analysis of TBSA in CSF by GLC-MS. (i) Technical equipment. TBSA analyses were done with a GLC-MS workstation (Hewlett Packard, Palo Alto, Calif.) which consisted of an HP 5890 II series gas chromatograph, an HP 5971 qua-

drupole mass-selective detector, and an HP 7673 autosampler. The column used was an ultra-performance capillary column (25 m by 0.2 mm by 0.33 μm) coated with cross-linked 5% phenylmethyl silicone (Ultra 2; Hewlett Packard). Helium was used as the carrier gas. At the beginning of the program the column pressure was set to 130 kPa (flow, 0.83 ml/min). The temperature was increased from 110 to 260°C at a rate of 6°C/min. Injector and detector temperatures were set to 260 and 200°C, respectively. MS analyses were carried out by single-ion monitoring at *m/e* 74, 167, and 312 (6, 14).

(ii) Sample preparation. Samples were prepared as described previously (14) with some modifications (11). Briefly, 0.5 ml of CSF was mixed with equal volumes of methanol and a 5% (wt/vol) aqueous solution of sodium hydroxide. The mixture was incubated at 100°C for 30 min and then acidified by adding 0.5 ml of 6 N hydrochloric acid. Extraction of the fatty acids was performed with 4 ml of hexane-chloroform (4:1, vol/vol). The organic layer was separated quantitatively, dried with sodium sulfate, and evaporated to dryness under a stream of N_2. Fatty acids were derivatized by adding 0.2 ml of 3 N methanolic hydrochloric acid. After 12 h at room temperature, the fatty-acid methyl esters were extracted with 4 ml of hexane. The hexane phase was separated quantitatively and evaporated to dryness (under N_2). For GLC-MS analysis the samples were dissolved in 0.1 ml of hexane. The injection size was 1 μl.

(iii) TBSA standards. Synthetic racemic TBSA (a gift from L. Larsson, University of Lund, Lund, Sweden) was used as a standard. TBSA was also extracted from cultures of *M. tuberculosis* ATCC 25618 and isolated as its methyl ester by preparative thin-layer chromatography (12). Signals at *m/z* 74, 167, and 312 were obtained by single-ion monitoring.

Patients' clinical data. In those cases in which discrepant results for MTD and culture were obtained, the physician in charge was contacted and the clinical data of the patients were evaluated. Clinical assessment included the patient's history, signs, symptoms, chest X ray, laboratory results, and follow-up observation as well as the results obtained with additional specimens from the patient that were sent to the mycobacteriology laboratory.

Statistical analyses. The chi-square test was done by using Epi Info (version 5.0; Centers for Disease Control and Prevention, Atlanta, Ga.). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MTD were calculated in comparison with culture results and, separately, in comparison with culture results plus the patients' clinical data.

RESULTS

Adaptation of MTD to clinical specimens of nonrespiratory origin: model study with CSF. Prior to being spiked with *M. tuberculosis* cells, all CSF specimens were tested for the possible presence of microorganisms. In none of the cases could AFB, other bacteria, or fungi be detected by microscopy or culture. Major standardization experiments with MTD or traditional techniques (culture, GLC-MS) were run in triplicate.

When suspended in phosphate buffer, fewer than 10 *M. tuberculosis* bacteria (which corresponds to ca. 2×10^2 cells per ml) per assay could easily be detected by MTD. Direct detection of *M. tuberculosis* with 50 μl of CSF specimen, however, yielded a positive result only when at least 5×10^5 cells per ml were present. Neither extensive washing of the CSF pellet with phosphate buffer nor precipitation of presumed interfering compounds by trichloroacetic acid improved test sensitivity. Addition of Tween 80 to the centrifuged CSF pellet was also not feasible, since Tween 80 alone, when subjected to MTD, caused consistently false-positive signals (89,254 to 343,821 RLU). Also, incubation of CSF spiked with *M. tuberculosis* cells with Fast Express (Gen-Probe) for 10 min at 60°C and 10 min at room temperature following sonication prior to amplification had no effect; i.e., values for RLU were always <30,000. Eventually, we succeeded in treating CSF with SDS-NaOH. Increasing the amount of sample 10-fold (500 μl instead of 50 μl) followed by centrifugation and increasing the amplification time from 2 to 3 h contributed, furthermore, to a significantly enhanced sensitivity of MTD. A denaturation time of 40 min with SDS-NaOH was mandatory since shorter interaction times resulted in a marked decrease in test sensitivity (10^2 cells per ml of CSF at 40 min interaction time versus 10^3 cells per ml of CSF at 5 min). All in all, standardization of MTD with artificially spiked CSF was done six times. The test was consistently positive for suspensions containing 10^2 cells of *M. tuberculosis* per ml (which corresponds to approximately 50 bacteria per assay). In most of the runs, positive MTD results were also obtained for more dilute suspensions (as few as ca. 5 cells per ml) (Table 2, footnote *b*).

TABLE 2. Conventional and molecular biological methods used for the detection of *M. tuberculosis* in CSF

Technique	No. of cells of *M. tuberculosis*/ml of CSF required for a positive result	Time required for detection
Microscopy	10^4–10^5	30 min
Löwenstein-Jensen, Middlebrook 7H10 and selective 7H11 culture	10^6	7–14 days
	10^3	21 days
BACTEC 12B culture	10^6	1 day
	10^4	11 days
	10^3	13 days
	5×10^2	15 days
	$<10^2$	NG[a]
GLC-MS	5×10^3–5×10^4	24 h
MTD	10^{2b}	8 h[c]

[a] NG, no growth after 8 weeks.

[b] A total of six runs were performed. MTD yielded positive results with 10^2 cells per ml (six times), 5×10^1 cells per ml (one time), 10^1 cells per ml (three times), and 0.5×10^1 cells per ml (two times).

[c] Time for pretreatment with SDS-NaOH included.

At least 10^4 to 10^5 AFB per ml had to be present in CSF for a positive result by microscopy. With these cell concentrations, colonies of *M. tuberculosis* were observed on solid media after, at the earliest, 7 to 14 days of incubation. In requiring at least 5×10^2 AFB per ml to be present in the initial inoculum, the radiometric method proved to be the most sensitive and rapid cultivation technique (15 days) (Table 2). When 12B medium was inoculated with $<10^2$ AFB per ml, BACTEC cultures remained negative. Although as little as 10 pg of TBSA (which corresponds to the amount of TBSA from approximately 5×10^2 *M. tuberculosis* cells [6]) can be detected by GLC-MS, at least 5×10^3 cells per ml had to be initially present in the CSF specimen to allow a quantitative extraction of TBSA and derivatization of its fatty acid to the methyl ester. Characteristic signals for TBSA were obtained at *m/z* 74, 167, and 312. When more dilute cell suspensions were analyzed, TBSA could not be detected (Table 2).

Performance of MTD in a clinical mycobacteriology laboratory. (i) RLU values. Positive and negative MTD results could be very clearly discriminated by the magnitude of the RLU value. For smear-positive, culture-positive specimens (*M. tuberculosis* complex), 88% of all values exceeded 2,000,000 RLU. Only 46% of smear-negative, culture-positive specimens exhibited values with greater magnitude, whereas 39% showed values of <1,000,000 RLU (range, 50,777 to 998,293 RLU). Negative results were usually far below the cutoff value of 30,000 RLU; 92.9% had values of <10,000 RLU.

(ii) Overall comparison of smear, culture, and MTD results. Of the 1,439 specimens tested, 69 yielded nontuberculous mycobacteria by conventional culture. In all these cases, MTD remained negative. Nontuberculous mycobacteria included the following *Mycobacterium* species (number of isolates in parentheses): *M. avium* complex (31), *M. kansasii* (10), *M. xenopi* (8), *M. gordonae* (5), *M. fortuitum* (4), *M. malmoense* (3), *M. haemophilum* (2, both of which grew radiometrically without hemin), *M. gordonae* plus *M. avium* complex (2), *M. chelonae* (1), *M. terrae* (1), *M. scrofulaceum* (1), and *M. kansasii* plus *M. avium* complex (1). Of all clinical specimens tested, 140 (56 smear-positive and 84 smear-negative) specimens were MTD positive and culture positive, and 1,206 specimens were nega-

tive by both methods. There were 93 discrepant results: 71 specimens were positive by MTD but negative by culture, and 22 specimens were negative by MTD but grew *M. tuberculosis* upon culture (Table 1). Overall sensitivity, specificity, PPV, and NPV of MTD were 86.4, 94.4, 66.4, and 98.2%, respectively. After a review of discrepant results, these values were adjusted to 88.5, 96.7, 80.6, and 98.2%, respectively (see Table 4).

(iii) Nonrespiratory specimens. Of the nonrespiratory specimens (n = 322), 39 were MTD and culture positive (*M. tuberculosis*) and 258 were negative by both methods. There were 25 discrepant results: 21 specimens were positive by MTD and negative by culture, and 4 were negative by MTD but positive by culture (Table 1). On the basis of these data, overall sensitivity of MTD for nonrespiratory specimens was 90.7%, specificity was 92.5%, PPV was 65.0%, and NPV was 98.5%.

Among the 54 CSF specimens obtained for MTD, there were six cases for which tuberculous meningitis was suspected on account of clinical symptoms and chemical parameters. The only smear-positive CSF specimen obtained yielded an RLU value of 3,031,180. Values for the smear-negative specimens were 2,794,178, 1,630,161, 1,352,634, 314,222, and 96,695 RLU. Except for the specimen exhibiting the lowest RLU value, positive radiometric cultures for *M. tuberculosis* were obtained (after 7 days for the smear-positive CSF specimen and after 3 to 6 weeks for the smear-negative CSF specimens). In only 2 of 6 CSF specimens was the concentration of TBSA high enough (>10 pg) to be unequivocally detected by GLC-MS. For the sixth patient (RLU value, 96,695) both culture and TBSA analysis of CSF were negative, but urine, BAL, lung biopsy, and blood specimens from this patient yielded *M. tuberculosis*, so this case was considered a true positive (Table 3). In two cases, though patients exhibited neurological symptoms, active TB could not be assessed: their positive MTD results were considered false positives.

As to the 53 urine specimens tested, 8 were MTD positive (RLU values between 130,293 and 3,153,174; 6 specimens had RLU values of >2,400,000) and culture positive. Of these eight specimens, five were smear positive (RLU range, 1,870,494 to 3,153,174) and three were smear negative (RLU range, 130,293 to 2,663,719). There were three false-positive results. One of them was negative upon retesting; the other two could, however, be considered true positives (Table 3), since one patient (HIV infected) had been diagnosed with disseminated TB 13 months earlier and had been receiving treatment for 9 months. In the other true-positive case, the patient's chest X ray showed extensive pulmonary cavities. MTD of the urine sample of the latter patient remained positive upon repeat, and an additional specimen (BAL) yielded a positive MTD as well. We failed, however, to cultivate *M. tuberculosis* in the respiratory specimen.

Of 46 lymph nodes examined, 12 were MTD positive and culture positive. Of them, six were smear positive (RLU range, 108,777 to 2,795,356) and six were smear negative (RLU range, 866,999 to 2,715,612). There were seven MTD-positive, culture-negative cases. They all yielded RLU values between 430,190 and 3,139,930. Six of them originated from patients with an actual or recent TB history and were therefore considered true positives (Table 3). Among them was one patient from whom *M. avium* complex in addition to *M. tuberculosis* could be isolated. The seventh lymph node specimen, which was MTD positive but culture negative, remained inconclusive. For this patient, TB had been suspected years ago but was never confirmed by culture.

Two pleural biopsies (of four) and 2 pleural aspirates (of 65) were MTD positive and culture positive. All were smear negative and exhibited more than 2,500,000 RLU. One MTD-positive, culture-negative aspirate was considered a true positive (Table 3) since at the same time a second pleural aspirate from the same patient yielded a positive culture. Two other specimens yielded RLU values which were slightly above the cutoff value of 30,000 RLU and negative upon retesting (see below). Two pleural aspirates yielded false-negative MTD results. All other pleural specimens were negative by both methods.

The majority of all other nonrespiratory specimens, such as gastric juice, pus, and the various types of biopsy samples and aspirates, showed MTD results which mainly agreed with culture results (Table 1). Concerning the total of nonrespiratory samples, 15 MTD-positive and culture-negative specimens were considered true positives and one case remained inconclusive (Table 3). There were three culture-negative specimens (two pleural aspirates, one urine sample) which yielded positive MTD results but yielded negative results upon retesting (not shown in Table 3). Nevertheless, they were considered false positives. False-negative results for all nonrespiratory specimens tested (MTD negative and culture positive for *M. tuberculosis*) were obtained in only four cases: two pleural aspirates (see above) and two pus specimens (all smear negative).

After a review of the patients' clinical data in those cases in which discrepant results were found, the adjusted sensitivity, specificity, PPV, and NPV of MTD for nonrespiratory specimens were determined to be 93.1, 97.7, 90.0, and 98.5%, respectively (Table 4). For smear-positive (n = 16) compared with smear-negative (n = 23) specimens, uncorrected values of MTD sensitivity were 100 and 85.2%, respectively, and the specificities were 100 and 92.5%, respectively. For smear-negative specimens, the corrected values of MTD sensitivity and specificity were 90.5 and 97.8%, respectively. We had three specimens with initial RLU values slightly above the cutoff that were negative upon repeat. Had these specimens been considered true negatives, the specificity of MTD for smear negatives would have risen to 93.8% before and 99.3% after resolution of discrepant results.

(iv) Respiratory specimens. Of the 1,117 respiratory specimens, 101 were MTD and culture positive and 948 were negative by both techniques. In total, there were 68 discrepant results in this group: 50 specimens were MTD positive but negative by culture, and for 18 specimens, all smear negative, the opposite held true (Table 1). On the basis of these data, sensitivity, specificity, PPV, and NPV of MTD were 84.9, 95.0, 66.9, and 98.1%, respectively. After the patients' clinical data were studied, 15 cases could be resolved. These specimens originated from patients who were still on anti-TB therapy. Although 13 other patients had respiratory symptoms, there were no signs of active pulmonary TB in this latter group. Apart from one inconclusive situation, we found 21 MTD-positive, culture-negative specimens. Nearly 70% of them exhibited RLU values of <100,000, and another 20% of them exhibited RLU values of <200,000. Upon repeat, MTD was clearly negative in all 21 cases. For our evaluation, these specimens were, nevertheless, considered false positives as well. After resolution of the discrepancies (analysis not shown; on special request, a summary can be obtained from the corresponding author), the sensitivity, specificity, PPV, and NPV of MTD increased to 86.6, 96.4, 76.8, and 98.1%, respectively). Distinguishing again between smear-positive (n = 40) and smear-negative (n = 61) samples, uncorrected values for MTD sensitivity were 100 and 77.2%, respectively. Specificities were 100 and 95.0%, respectively. For smear-negative samples, the corrected values for sensitivity and specificity were 80.9 and

TABLE 3. Analysis of discrepant results between MTD-positive and culture-negative cases (nonrespiratory specimens)

Specimen type and no.	Patient no.	Patient's age (yr), sex[a]	No. of positive cultures/ no. of specimens tested[b]	Origin of specimen	Clinical diagnosis[c]	Comments	History of previous TB	Final interpretation of MTD test[d]
CSF								
1	10480/94	74, F	0/4	CSF	Purulent meningoencephalitis and pyelonephritis, septicemia	No evidence of tuberculous meningitis; positive MTD not explained	No	False +
2	11317/94	87, M	0/6	CSF	Dementia	Pulmonary TB in 1967; at present, no signs, symptoms, or findings indicating active TB, particularly no evidence of meningoencephalitis	Yes	False +
3	5499/95[e]	37, F	4/5	CSF	TB meningitis, somnolence, fever	HIV infection; disseminated TB; multiple positive cultures (BAL, urine, blood, lymph node)	No	True +
Lymph node								
4	5582/94	31, F	0/1	Lymph node biopsy	Active cervical tuberculous lymphadenitis, pulmonary TB	Pregnant woman	No	True +
5	5120/95	32, F	0/2	Lymph node biopsy	Cervical lymphadenitis	TB 7 yr ago, never confirmed by culture; TB treatment probably insufficient	Yes	Inconclusive
6	1000/95	28, F	2/7	Cervical lymph node biopsy	TB of cervical lymph node	Smear-positive pulmonary TB 1 yr ago, treated for 6 mo, patient compliant; few AFB in a fine-needle aspirate	Yes	True +
7	10175/94	40, M	0/3	Cervical lymph node biopsy	TB of cervical lymph node	Typical clinical presentation; histology: granulomatous inflammation; TB treatment for 9 mo	No	True +
8	4192/95	57, M	0/6	Hilar lymph node biopsy	Pulmonary TB	Reactivation of TB; TB treatment initiated	Yes	True +
9	5487/95[e]	37, F	4/5	Hilar lymph node biopsy	Disseminated TB	HIV infection; multiple positive TB cultures (BAL, urine, blood)	No	True +
10	4350/95	15, M	0/12	Lymph node biopsy	TB of supraclavicular lymph node	Four lymph node specimens with AFB (microscopically determined); PCR inhibited	Mother has TB	True +
Urine								
11	6740/94	44, M	5/40	Urine	Cough; assumed reactivation of TB	HIV infection, CD4 cells 0.03 $\times 10^9$/liter; disseminated TB diagnosed 13 mo ago, treated for 9 mo	Yes	True +
12	3397/95	25, M	0/5	Urine	Pulmonary and renal TB	Chest X ray: cavitation in right upper lobe; signs and symptoms suggestive of TB	No	True +
Other nonrespiratory specimens								
13	10579/93	87, M	0/2	Aspirate of psoas abscess	Spondylitis, paraspinal abscess	Bacterial cultures also negative; TB treatment initiated	No	True +
14	2549/94	76, M	0/1	Aspirate of pericardial effusion	Pericardial and pleural effusion of unknown origin	TB treatment initiated	No	True +
15	9588/93	68, M	0/9	Spinal bone biopsy	Spondylitis	Signs and symptoms suggestive of tuberculous spondylitis; TB treatment initiated	No	True +
16	552/94	47, M	2/6	Aspirate of pleural effusion	Pleuritis tuberculosa	Histology: granulomatous inflammation; X ray suggestive of TB	Father died of TB	True +

Continued on following page

TABLE 3—*Continued*

Specimen type and no.	Patient no.	Patient's age (yr), sex[a]	No. of positive cultures/ no. of specimens tested[b]	Origin of specimen	Clinical diagnosis[c]	Comments	History of previous TB	Final interpretation of MTD test[d]
17	5343/95[f]	30, M	2/9	Pannus biopsy	Skin TB	HIV infection since 1985; no signs of lung TB, positive cultures from skin specimens; TB treatment initiated	No	True +
18	5344/95[f]	30, M	2/9	Pannus biopsy	Skin TB	As above	No	True +

[a] F, female; M, male.
[b] Specimens tested were not all necessarily included in the study period.
[c] Assessment based on signs, symptoms, laboratory results, chest X ray, results of TB cultures, and follow-up.
[d] False +, false positive; True +, true positive.
[e] 5499/95 and 5487/95 represent the same patient.
[f] 5343/95 and 5344/95 represent the same patient.

96.5%, respectively. Eliminating, again, the 20 respiratory specimens which showed RLU values slightly above the cutoff and became negative upon retesting, specificity of MTD would have risen to 96.9% before and 98.5% after resolution of discrepant results.

(v) Nonrespiratory versus respiratory specimens. There were no statistically significant differences in sensitivity and specificity between respiratory and nonrespiratory specimens, neither before nor after resolution of discrepancies ($P > 0.005$).

DISCUSSION

Nucleic acid amplification procedures have brought considerable impact on the rapid detection of TB. Regardless of whether PCR or transcription-mediated amplification technology is applied, the clinical usefulness of these methods for some situations remains, however, biased. In particular, this holds for smear-positive sputum specimens, for which up to 96% of patients with pulmonary TB may be detected if more than one respiratory tract specimen is submitted to the laboratory (15). Even if *M. avium* complex predominates a clinical setting because of a large number of HIV-infected individuals, the high predictive value of the sputum smear remains practically unchanged (28). In contrast, infection with low numbers of bacilli, as frequently seen in extrapulmonary manifestations of TB, represents a clinical situation in which both an early and sensitive diagnosis of the disease is of utmost importance, thus prompting adaptation of nucleic amplification assays to nonrespiratory specimens. The facts that extrapulmonary presentations are approximately three times less frequently encountered than lung TB (7) and that nonrespiratory specimen collection can be more difficult may explain why nonrespiratory specimens have hitherto been neglected in studies using amplification-based detection of *M. tuberculosis.*

Using human CSF which has been artificially spiked with defined amounts of *M. tuberculosis* cells, we have simulated the clinical situation of tuberculous meningitis with respect to bacterial concentration. With this model system we were able to establish MTD for CSF and compare its performance with that of radiometric culture and direct detection of TBSA by GLC-MS. When we subjected real CSF to MTD, surprisingly high amounts of cells were necessary for a positive result, suggesting interference of unknown compounds with the components of the assay. The phenomenon of interference in amplification-based techniques is not uncommon: pus samples, tissue biopsy specimens, and sputa were found to be up to 20% inhibitory in PCR-based systems (2, 13). Similarly, Ehlers et al. (4) have observed complete inhibition of the MTD when applied to a CSF and a pleural aspirate specimen spiked with *M. bovis* BCG cells. Very recently, Vlaspolder et al. (26) have reported an unacceptable sensitivity of 20% for pleural exudates compared with culture (on Löwenstein-Jensen slants only). To finally establish MTD for CSF, three modifications were made: (i) the amount of sample was increased 10-fold (500 μl instead of 50 μl), (ii) pretreatment with a denaturing agent such as SDS was

TABLE 4. MTD compared with culture and clinical assessment of patients[a]

Type of specimen and MTD result	No. of cases with clinical diagnosis of TB		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
Nonrespiratory plus respiratory (n = 1,439)			88.5	96.7	80.6	98.2
Positive	170	41				
Negative	22	1,206				
Nonrespiratory (n = 322)			93.1[b]	97.7[b]	90.0	98.5
Positive	54	6				
Negative	4	258				
Respiratory (n = 1,117)			86.6[b]	96.4[b]	76.8	98.1
Positive	116	35				
Negative	18	948				

[a] Values in this table are based on data after resolution of discrepant results.
[b] No statistically significant difference (chi-square test).

performed, and (iii) the amplification time was increased from 2 to 3 h. With these adjustments, we were consistently able to detect ca. 10^2 cells per ml by MTD. Mostly, positive results were also attained with more dilute cell suspensions (with as little as 5 cells per ml [Table 2, footnote *b*]). These variable results clearly illustrate the sampling problem commonly encountered with extremely small loads of TB bacilli as a result of their tendency to clump together (27). Increasing the initial amount of specimen from 50 to 500 μl and subjecting the specimen to a subsequent centrifugation allows MTD an increased amount of target for amplification. SDS, on the other hand, a detergent which denatures proteins and enzymes (20), obviously eliminates most of the inhibitory effects present in CSF. It has been shown earlier that our pretreatment protocol with SDS contains sufficient washing steps to remove any traces of the detergent which might interact with the assay system (19). With a very short turnaround time and an excellent sensitivity and specificity, MTD is, thus, far better than both culture and analysis for TBSA detection. For AFB concentrations of 10^2 to 10^3/ml, CSF usually took at least 15 days until radiometric cultures became positive, while comparable cultures on solid media needed 3 to 4 weeks for positivity. GLC-MS represents a valuable tool in the rapid diagnosis of tuberculous meningitis (<24 h), but only as long as TBSA levels amount to more than 10 pg (equivalent to at least 5×10^3 to 5×10^4 AFB). Since these concentrations may very rarely be attained in early stages of tuberculous meningitis, the method evidently gives rise to misleading, false-negative results. Other drawbacks of this technique are that (i) with the present setup only 1/100 of the initial 500 μl of CSF used for extraction and derivatization can eventually be injected into the GLC-MS instrument, (ii) TBSA is not an exclusive characteristic of *M. tuberculosis* but is also a membrane constituent of most nontuberculous mycobacteria and some gram-positive rods (14), and (iii) the technique is both financially and operationally demanding. In total, we have diagnosed six cases of tuberculous meningitis by MTD. Even though positive cultures were obtained radiometrically from only five of the six CSF specimens (smear positive, 1; smear negative, 5), the remaining case undoubtedly represented a true positive, since the patient had disseminated TB as indicated by the isolation of *M. tuberculosis* from lymph node, blood, urine, and respiratory specimens. That positive cultures were only obtained in five cases and TBSA was detected in merely two of these patients demonstrates the difficulties and limitations in diagnosing tuberculous meningitis by present-day traditional microbiological techniques and by TBSA analysis. The fact that the neurological status of the patient with MTD-positive but culture-negative CSF specimen had considerably improved after the onset of antituberculous chemotherapy points unambiguously to the correctness of the MTD result.

As for nonrespiratory specimens other than CSF, MTD worked well with urine and gastric-fluid specimens, and none of the above-mentioned difficulties were encountered with various types of biopsy samples and most aspirates or with lymph nodes. For smear-positive urine or lymph node specimens, pretreatment with SDS is not even mandatory. For the sake of feasibility and simplicity in a routine laboratory, however, it appears easier to pretreat all specimens in the same way. A hemorrhagic or very viscous consistency of the specimen, as is frequently observed with pericardial and pleural aspirates, can give rise to high background levels (RLU values around the cutoff value) and may, thus, be a potential cause for false-positive results.

Our study, encompassing more than 320 nonrespiratory specimens, demonstrates that MTD is also highly sensitive and specific for these kinds of clinical samples (Table 4). We obtained a total of four false-negative MTD results from two pus specimens and two pleural aspirates. Difficulties of MTD in detecting *M. tuberculosis* in pleural specimens are well known. When 61 specimens were pretreated with *N*-acetyl-L-cysteine, 5 yielded positive cultures, and only one single aspirate thereof was positive by MTD; thus, test sensitivity did not exceed 20% (26). Although the number of pleural exudates we tested is likewise low ($n = 65$), we observed a better sensitivity (50%) when pleural aspirates were pretreated with SDS-NaOH. However, this value is still far from being satisfactory. Of the total 21 false-positive results observed among the nonrespiratory specimens, 3 were considered false positive despite negative results upon retesting (not shown in Table 3). Of the 18 remaining cases, 15 discrepant cases could be resolved, 1 case was inconclusive, and the remaining 2 cases were considered false positive since follow-up observation of the patients, though very brief, has so far not suggested the presence of active TB (Table 3). MTD detected all 15 smear-positive, nonrespiratory specimens and 23 (85.2%) of the smear-negative specimens which were culture positive. Taking into account MTD-positive, culture-negative specimens which turned out to be true positives (Table 3), 90.5% of all smear-negative samples could be detected by MTD, demonstrating that this test exhibits a high sensitivity for smear-negative extrapulmonary specimens, in particular compared with the culture which includes an egg- and agar-based as well as a liquid medium as the cultural "gold standard."

As for the 1,117 respiratory specimens tested over a 2-year period, the specificity, PPV, and NPV are similar to earlier results (19). The sensitivity of 86.6% (Table 4) is, however, lower than that which we have observed before (97.4% [19]) but is still in the range reported by others (1, 8, 16). The lower sensitivity could be attributed to the real, i.e., nonevaluation, situation in a clinical mycobacteriology laboratory, which means that, normally, MTD is performed by different technicians and the number of specimens to be tested may vary considerably (in our laboratory, between 5 and 45 per run).

False negatives are most likely due to sampling errors (nonuniform distribution of microorganisms), to the presence of possible amplification inhibitors in the samples, or to low RNA levels due to poor viability of mycobacteria, as demonstrated by the unusually long period until detection in culture (6 to 8 weeks) with some of the specimens. Of the 1,117 respiratory specimens tested, 50 had positive MTD results but negative cultures. Of these, 15 turned out to be true positives (Table 4) while the other 35 were considered false positives. Among the latter, more than 70% exhibited RLU values slightly above the cutoff and were true negatives upon retesting. This demonstrates that the cutoff (proposed value, 30,000 RLU) should probably be redefined as a zone. Good laboratory practice must aim at repeating all MTDs which yield RLU values between >30,000 and ca. 200,000. With this approach, 24 false-positive results could have been avoided. Possible explanations for the remaining false-positive results may be unknown technical problems, unless TB can be verified in follow-ups of one patient or the other.

When sensitivity, specificity, PPV, and NPV of MTD are compared, after resolution of the discrepancies, performance characteristics are even better with nonrespiratory specimens (93.1, 97.7, 90.0, and 98.5% respectively) than with respiratory specimens (86.6, 96.4, 76.8, and 98.1%, respectively). The differences in sensitivity and specificity of these two groups of specimens are, however, not statistically significant ($P > 0.05$ [chi-square test]). Although MTD sensitivity is superior to that of the cultural approach (culture sensitivities were 90% for

nonrespiratory and 76.8% for respiratory specimens versus 93.1 and 86.6%, respectively, for MTD) and MTD provides both the laboratory and the clinician with the most rapid result, neither MTD nor any of the currently available kit-based amplification techniques free the laboratory from establishing the labor-intensive procedure of culture since (i) all nontuberculous mycobacteria would be missed, (ii) members within the *M. tuberculosis* complex cannot be differentiated, and (iii) biomass is still mandatory for routine susceptibility testing despite progress in the molecular understanding and rapid detection of resistance against primary anti-TB agents (29). Therefore, any amplification-based test for direct detection can, at present, only be used as an adjunct to conventional standard procedures. Nevertheless, as demonstrated here, MTD can provide rapid, reliable, and accurate results, in particular for difficult cases. For optimum results, however, a close cooperation between the clinician and the laboratory is needed to define those specimens which urgently have to be screened by MTD, i.e., those cases in which the clinical usefulness of MTD is justified.

ACKNOWLEDGMENTS

We thank A. von Graevenitz and C. Adler for helpful suggestions. P. Pei, Department of Clinical Chemistry, University of Zurich, provided CSF samples, and Gen-Probe provided MTD kits for the analysis of CSF specimens.

This work was supported by the Swiss Federal Office of Public Health and the Zürcher Lungenliga.

REFERENCES

1. **Abe, C., K. Hirano, M. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and S. Goto.** 1993. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J. Clin. Microbiol. **31:** 3270–3274.
2. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and B. B. Plikaytis.** 1993. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol. **31:**2049–2056.
3. **D'Amato, R. F., A. A. Wallman, L. H. Hochstein, P. M. Colaninno, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kim, R. C. Patel, and A. Miller.** 1995. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. J. Clin. Microbiol. **33:**1832–1834.
4. **Ehlers, S., M. Pirmann, W. Zaki, and H. Hahn.** 1994. Evaluation of a commercial rRNA target amplification assay for detection of *Mycobacterium tuberculosis* complex in respiratory specimens. Eur. J. Clin. Microbiol. Infect. Dis. **13:**827–829.
5. **Fauville-Dufaux, M., B. Vanfleteren, L. De Wit, J. P. Vincke, J. P. Van Vooren, M. D. Yates, E. Serruys, and J. Content.** 1992. Rapid identification of tuberculous and non-tuberculous mycobacteria by polymerase chain reaction amplification of a 162 bp DNA fragment from antigen 85. Eur. J. Clin. Microbiol. Infect. Dis. **11:**797–803.
6. **French, G. L., C. J. Chan, R. Teoh, M. J. Humphries, and G. O'Mahony.** 1987. Diagnosis of tuberculous meningitis by detection of tuberculostearic acid in cerebrospinal fluid. Lancet **ii:**117–119.
7. **Humphries, M. J., W. K. Lam, and R. Teoh.** 1994. Non-respiratory tuberculosis, p. 93–125. *In* P. D. O. Davies (ed.), Clinical tuberculosis. Chapman and Hall Medical, London.
8. **Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
9. **Kennedy, D. H., and R. J. Fallon.** 1979. Tuberculous meningitis. JAMA **241:**264–268.
10. **Kent, P. T., and G. Kubica.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Public Health Service, U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta.
11. **Kissling, P., C. Adler, and G. E. Pfyffer.** 1994. Rapid detection of acid-fast bacilli in cerebrospinal fluid: gas liquid chromatography/mass spectrometry *vs* Gen-Probe Amplified *M. tuberculosis* Direct Test, abstr. U-8, p. 174. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
12. **Knisley, C. V., J. J. Damato, J. K. McClatchy, and P. J. Brennan.** 1985. Rapid and sensitive identification of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **22:**761–767.
13. **Kolk, A. H. J., A. R. J. Schuitema, S. Kuijper, J. van Leeuwen, P. W. M. Hermans, J. D. A. van Embden, and R. A. Hartskeerl.** 1992. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. J. Clin. Microbiol. **30:**2567–2575.
14. **Larsson, L., P. A. Mardh, and G. Odham.** 1979. Detection of tuberculostearic acid in mycobacteria and nocardiae by gas-chromatography and mass spectrometry using selected ion monitoring. J. Chromatogr. **163:**221–224.
15. **Lipsky, B. A., J. Gates, F. C. Tenover, and J. J. Plorde.** 1984. Factors affecting the clinical value of microscopy for acid-fast bacilli. Rev. Infect. Dis. **6:**214–222.
16. **Miller, N., S. G. Hernandez, and T. J. Cleary.** 1994. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol. **32:**393–397.
17. **Miyazaki, Y., H. Koga, S. Kohno, and M. Kaku.** 1993. Nested polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **31:**2228–2232.
18. **Nolte, F. S., and B. Metchock.** 1995. Mycobacterium, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology Press, Washington, D.C.
19. **Pfyffer, G. E., P. Kissling, R. Wirth, and R. Weber.** 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J. Clin. Microbiol. **32:**918–923.
20. **Reynolds, J. A., and C. Tanford.** 1970. Binding of dodecyl sulphate to proteins at high binding ratios: possible implications for the state of proteins in biological membranes. Proc. Natl. Acad. Sci. USA **66:**1002–1007.
21. **Salfinger, M., and G. E. Pfyffer.** 1994. The new mycobacteriology laboratory. Eur. J. Clin. Microbiol. Infect. Dis. **13:**961–979.
22. **Schöningh, R., C. P. H. J. Verstijnen, S. Kuijper, and A. H. J. Kolk.** 1990. Enzyme immunoassay for identification of heat-killed mycobacteria belonging to the *Mycobacterium tuberculosis* and *Mycobacterium avium* complexes and derived from early cultures. J. Clin. Microbiol. **28:**708–713.
23. **Shinnick, T. M., and V. Jonas.** 1994. Molecular approaches to the diagnosis of tuberculosis, p. 517–530. *In* B. R. Bloom (ed.), Tuberculosis. American Society for Microbiology Press, Washington, D.C.
24. **Smid, I., and M. Salfinger.** 1994. Mycobacterial identification by computer-aided gas-liquid chromatography. Diagn. Microbiol. Infect. Dis. **19:**81–88.
25. **Tevere, V., M. D. Appleman, M. Bankowski, W. Black, R. D'Amato, M. Delvecchio, R. Gander, L. Heifets, P. Hopewell, B. Metchock, D. Moore, K. Smith, and D. Jungkind.** 1995. Results of a multicenter clinical trial study for the direct detection of *Mycobacterium tuberculosis* in respiratory samples utilizing the Amplicor™ *M. tuberculosis* test, abstr. U-91, p. 132. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
26. **Vlaspolder, F., P. Singer, and C. Roggeveen.** 1995. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. J. Clin. Microbiol. **33:**2699–2703.
27. **Wayne, L. G.** 1994. Cultivation of *Mycobacterium tuberculosis* for research purposes, p. 73–83. *In* B. R. Bloom (ed.), Tuberculosis. American Society for Microbiology Press, Washington, D.C.
28. **Yajko, D. M., P. S. Nassos, C. A. Sanders, J. J. Madej, and W. K. Hadley.** 1994. High predictive value of the acid-fast smear for *Mycobacterium tuberculosis* despite the high prevalence of *Mycobacterium avium* complex in respiratory specimens. Clin. Infect. Dis. **19:**334–336.
29. **Zhang, Y., and D. Young.** 1994. Molecular genetics of drug resistance in *Mycobacterium tuberculosis*. J. Antimicrob. Chemother. **34:**313–319.